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Effects of $I_{k,Ach}$ Channel Inhibitor Tertiapin-Q on Right Atrial Preparations from Patients in Sinus Rhythm and Atrial Fibrillation

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Background: The acetylcholine-activated inward rectifying current, $I_{K,Ach}$, has been suggested as a novel atrial-selective putative drug target for the treatment of atrial fibrillation (AF). Here we have studied the effects of a selective $I_{K,ACh}$ channel inhibitor tertiapin-Q in human right atrial preparations from patients in sinus rhythm (SR) and permanent chronic atrial fibrillation (AF, >6 months).

Methods: Action potentials (APs) were measured using sharp electrodes in right atrial trabeculae or patch electrodes in cardiomyocytes from patients undergoing open heart surgery (48 in SR and 34 in AF).

Results and Conclusion: Tertiapin-Q (300nM) did not affect APs recorded in AF trabeculae (APD_{90} at 1Hz: control 205.8 ± 9.0ms *vs.* drug 209.0 ± 9.0ms, n.s.). Consistent with electrical remodelling of $I_{K,ACh}$ channels in AF, 1μM carbachol caused minimal effect on APD in AF trabeculae and no further effect was detected following combined exposure to carbachol and tertiapin-Q (APD_{90} 242.0 ± 16.9ms, 233.0 ± 20.5ms, 234.0 ± 19.1ms, respectively). In SR trabeculae, tertiapin-Q modestly, though not significantly, attenuated the shortening effect of carbachol on APD_{90}: 310.0 ± 7.7ms, 215.0 ± 16.4ms, and 254.0 ± 17.8ms, respectively). In the continuous presence of carbachol and tetrodotoxin (1μM), tertiapin-Q prolonged effective refractory period and APD_{90}. In atrial cardiomyocytes from SR patients, tertiapin-Q fully reversed the carbachol-induced shortening of APD_{90}. At a holding potential of −80mV, tertiapin-Q had no effect on the basal inward current in SR cardiomyocytes (2.0 ± 0.5pA/pF vs. 2.5 ± 0.7pA/pF, n=7), but significantly reduced in AF cardiomyocytes (6.8 ± 1.1pA/pF vs. 5.9 ± 0.8pA/pF, $P<0.05$, n=12), providing evidence for constitutive activity of $I_{K,ACh}$ in AF cardiomyocytes. From the modest effects of tertiapin-Q in multicellular preparations in comparison to cardiomyocytes, we conclude that access of the $I_{K,ACh}$ channel inhibitor to its target may be limited in intact superfused tissues.

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Modulation of Pancreatic Islet Electrophysiology and Insulin Release by Potassium Channel Subunit Kvbeta2

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Voltage-gated potassium channels (Kv) regulate pancreatic β-cell excitability, and thereby insulin secretion. Kv channels are modulated by ancillary cytoplasmic subunits, such as the Kvβ proteins. In this study, we investigated the electrophysiology of pancreatic β-cells and isolated islet function in mice lacking the Kvβ2 gene.

Methods: Islets from $Kv\beta2^{-/-}$ (KO) and wild-type (WT) mice were enzymatically isolated and disrupted into single cells. Whole-cell voltage clamp was used to measure Kv kinetics. Action potentials were recorded using the perforated-patch technique. Glucose stimulated insulin secretion was measured in isolated WT and KO islets. Mice underwent metabolic characterization.

Results: Kvβ2 expression in WT islets was demonstrated by qRT-PCR and Western. Compared with cells from WT mice, KO β-cells had a depolarized half-activation voltage ($V_{1/2\text{-act}}$), $p<0.01$. The Kv-blocker 4-aminopyridine (4AP) was used to characterize the Kv current. The IC50 of the 4AP-sensitive current was 190μM. Total β-cell Kv amplitude blocked by 4AP was 34.1 ± 2.6% at 500μM. Together, this suggests Kv1-family proteins may contribute significantly to β-cell repolarization. Kvβ2 is known to modulate Kv1-family currents by increasing surface expression, shifting $V_{1/2\text{-act}}$, and altering channel inactivation. Insulin release from KO islets at 2.5mM glucose, 3.26 ± 0.25 ng/mL/hr/5 islets, was greater than in WT islets, 0.64 ± 0.23 ng/mL/hr, $p=0.002$. Fasting plasma insulin was higher in KO than in WT, $p<0.025$, and KO had lower fasting blood glucose, $p<0.0001$. An intraperitoneal injection of glucose showed KO to have enhanced glucose tolerance as compared to WT. When housed in metabolic cages, KO mice had higher metabolic rates than age-matched WT.

Conclusion: Deletion of Kvβ2 alters the metabolic phenotype and may be related to increased release of insulin by the murine pancreas due to altered β-cell repolarization. These findings reveal a critical role of Kvβ2 in regulating insulin release.

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Pharmacological Consequences of PKC Inhibition on Kv1.5+Kvbeta1.3 Channels

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The $K_v\beta1.3$ subunit modifies the gating and pharmacology of $K_v1.5$ channels in a PKC-dependent manner, decreasing channel sensitivity to bupivacaine- and quinidine-mediated blockade. Cardiac $K_v1.5$ channels associate with receptor for activated C kinase 1 (RACK1), the $K_v\beta1.3$ subunit and different PKC isoforms, resulting in the formation of a functional channelosome. The aim of the present study was to investigate the effects of PKC inhibition on bupivacaine and quinidine block of $K_v1.5 + K_v\beta1.3$ channels. HEK293 cells were transfected with $K_v1.5 + K_v\beta1.3$ channels, and currents were recorded using the whole-cell configuration of the patch-clamp technique. PKC inhibition was achieved by incubating the cells with either calphostin C or bisindolylmaleimide II and the effects of bupivacaine and quinidine were analysed. The voltage-dependent inactivation of $K_v1.5 + K_v\beta1.3$ channels and their pharmacological behavior after PKC inhibition with calphostin C were similar to those displayed by $K_v1.5$ channels alone. Indeed, the IC_{50} values for bupivacaine were similar in cells whose PKC was inhibited with calphostin C or bisindolylmaleimide II. Similar results were also observed in the presence of quinidine. The finding that the voltage-dependence of inactivation and the pharmacology of $K_v1.5 + K_v\beta1.3$ channels after PKC inhibition resembled that observed in $K_v1.5$ channels suggests that both processes are dependent on PKC-mediated phosphorylation. These results may have clinical relevance in diseases that are characterized by alterations in kinase activity. Supported by SAF2010-14916, SAF2013-45800-R and FIS-RIC RD12/0042/0019 Grants.

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Intersubunit Interactions Control Kir Channel Inactivation

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Inward rectifier potassium (Kir) channels are expressed in numerous mammalian tissues including the pancreas, brain, heart, and skeletal muscle and play a critical role in controlling cellular excitability. Pancreatic ATP-sensitive Kir (KATP) channels are key regulators of insulin secretion as they link cellular metabolism with membrane excitability. Loss-of-function (LOF) mutations in KATP can cause human hyperinsulinism as a result of diminished activity. Several of these LOF mutations disrupt salt-bridge interactions that are located within the intracellular subunit-subunit interfaces, and result in channel activity showing a fast inactivation following ATP removal as measured with the inside-out patch clamp technique. Inactivation can be subsequently abolished by application of PIP2 to the cytoplasmic face of the membrane, an action that can be explained by a simple model in which PIP2 competes with the closed inactivated state. We find that homologous mutations in the strong inward rectifying Kir2.1 channel cause lower basal activity as a result of reduced apparent PIP2 sensitivity, indicating increased inactivation. Kir2.1 channels contain additional intersubunit salt-bridge interactions that are not present in KATP channels. Introduction of these salt-bridges into the inactivating KATP channel mutants partially rescues the channel from this inactivating phenotype. These results lead us to propose that the stability of the intersubunit interface regulates channel inactivation, sensitivity to PIP2, and is conserved across the Kir channel family and stabilizing the intersubunit interface provides a potential strategy to exploit in development of activating modulators of KATP and other Kir channels

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Inhibition of Hsp70 Enhances A-Type Kv4 Current by Reducing Degradation of Auxiliary KChP4A

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We have previously shown that KChIP4s reduces surface expression of Kv4 through its N-terminal KID (Kv4 inhibitory domain) that causes endoplasmic reticulum retention of the channel complex. In this study we found that treatment of HEK 293 cells expressing KChIP4a with cycloheximide that inhibits protein synthesis causes a significant degradation of

KChIP4a. This accelerated degradation of KChIP4a was reversed by application of a proteasome inhibitor MG132, indicating the degradation of KChIP4a proteins through proteasome pathway. Functional dissection revealed a key domain consisting of eight hydrophobic and aliphatic residues in the N-terminus that is critical for degradation. Using mass spectrum analysis and co-immunoprecipitation assay, we further identified Hsp70 protein (heat shock protein 70) that can specifically interact with auxiliary KChIP4a. Inhibition of Hsp70 function by inhibitors Pifithrin-μ and VER-155008 can recover the reduction of surface Kv4 channels induced by KChIP4a, suggesting that Hsp70 is necessary for degradation of KChIP4a. In hippocampal neurons, inhibition of Hsp70 resulted in an increase of A-type current, suggesting a role of Hsp70 in functional Kv4 channel complexes. Further investigations of interactions between Hsp70 and KChIP4a and in vivo effects of Hsp70 inhibition on neurological functions in animal models are currently underway.

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Dynamic Subunit Stoichiometry of Kv4.3-KChIP4A Channel Complexes Visualized by Single-Molecule Subunit Counting

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Auxiliary Kv channel-interacting proteins 1-4 (KChIPs1-4) co-assemble with pore-forming Kv4 α-subunits to underlie somatodendritic subthreshold A-type current that regulates neuronal excitability. It has been hypothesized that different KChIPs can competitively bind to Kv4 α-subunit to form dynamic channel complexes that can exhibit distinct biophysical properties for modulation of neural function. To test this hypothesis, we utilized single molecule subunit counting to investigate whether different isoforms of auxiliary KChIPs such as KChIP4a and KChIP4bl can compete for binding of Kv4.3 to co-assemble hetero-multimeric channel complexes. Single-molecule imaging subunit counting revealed that the number of KChIP4 proteins in Kv4.3-KChIP channel complexes can vary depending on KChIP4 expression level. Increasing amount of KChIP4bl gradually reduces the bleaching steps of GFP for KChIP4a proteins and vice-versa. To further demonstrate Kv4 gating affected by different KChIP4 subunit stoichiometry, we generated two tandem constructs to mimic the situations of KChIP4a half-occupied channel complexes (KChIP4a-2xKv4.3) and KChIP4a saturated channel complexes (KChIP4a-Kv4.3) expressed in Xenopus oocytes. Gating kinetics of KChIP4a-2xKv4.3 co-expressed with KChIP4bl (to mimic channel complex like Kv4.3:KChIP4a:KChIP4bl with the ratio of 4:2:2) shows that both KChIP4a and KChIP4bl can simultaneously modulate the function of channel complexes upon co-assembly. The significance of dynamic channel complexes was further investigated in hippocampal neurons from kainic acid seizure model in rats by detecting a shift of expression profile from KChIP4bl to KChIP4a. Our preliminary findings demonstrate that auxiliary KChIPs can hetero-assemble with Kv4 in a competitive manner to form hetero-multimeric Kv4-KChIP4 complexes that are biophysically distinct and dynamically regulated under pathological conditions.

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The N-Terminal Extension of KChIP3 is Responsible for KChIP3-Calmodulin Complex Formation

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Potassium channel interacting proteins (KChIPs), belong to the family of neuronal calcium sensors that are expressed in brain, lung and heart tissue. KChIPs bind to Kv4 channels and regulate channel trafficking, membrane association, and current kinetics. Among them, KChIP-3, also known as Downstream Regulatory Element Antagonistic Modulator (DREAM) and calsenilin, interacts with other intracellular partners (presenilin, calmodulin, and DNA) and was implied in Alzheimer's disease and pain sensing, although a molecular mechanism of KChIP-3 interactions with distinct intracellular partners remains unknown. The objective of this study is to provide a molecular insight into the mechanism of KChIP-3 interactions with calmodulin and determine the role of the N-terminus of KChIP-3 in the formation of calmodulin:KChIP-3 complex. Full length KChIP-3 (residue 1-256) and ΔN KChIP-3 (residue 65-256) were over-expressed in *E. coli* and purified according to an established protocol. In addition, a peptide that corresponds to residues 29-44 in KChIP-3 N-terminus was synthesized. The equilibrium dissociation constants and rotational correlation times for calmodulin: KChIP-3 complexes were determined using fluorescence anisotropy. Molecular dynamic simulations were implemented to provide an insight into the potential molecular structure of the calmodulin:KChIP-3 protein complex. Calmodulin interaction with KChIP-3 shows a K_d=3.3 μM whereas the peptide analogous to KChIP-3(29-44) binds with K_d=150 nM, deletion of 64 residues from the KChIP-3 N-terminus abolishes the complex formation. The interactions between KChIP-3 and calmodulin are regulated by the presence of Ca^{2+}. A rotational correlation time of 35ns was determined for the KChIP-3:calmodulin complex, in agreement with a heterodimer of ellipsoidal shape. These results show that interactions between calmodulin and KChIP-3 are controlled by the intracellular calcium concentration and that the N-terminal extension in KChIP-3 provides a binding interface for calmodulin.

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Exploring Molecular Mechanisms of the Functional Interaction between Kv1.3 and Nav Beta1

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Voltage gated ion channels (VGICs) play crucial roles in the propagation electrical signals in excitable cells. In contrast, several VGICs have been also known to express in non-excitable cells and participate in keeping cell homeostasis. The voltage-gated potassium channel subfamily A member 3 (Kv1.3) is one of the Shaker-related channels expressed mainly on T lymphocytes. Many reports have indicated that Kv1.3 is associated with several diseases including autoimmune diseases, inflammatory diseases and obesity. On the other hand, the voltage-gated sodium channel (Nav) Beta 1 is known as a subunit modulating kinetics and expression of Nav, but several studies have shown that NavBeta1 also participates in brain development and cell signaling. These 'non-canonical' functions in VGICs have been focused upon recently and an exciting report showed the functional interaction between murine Kv1.3 (mKv1.3) and rat Navbeta1 (rBeta1) (Nguyan HM et al. PNAS 2012). The authors demonstrated that the activation of mKv1.3 ionic current is accelerated by rBeta1. Here, we explore the molecular mechanisms of the acceleration in Kv1.3 activation through gating currents of human Kv1.3 (hKv1.3) with and without rBeta1 using the cut-open voltage clamp technique. We found that the N-terminus of hKv1.3 is associated with trafficking efficiency of the protein to the membrane in Xenopus oocytes. Our data indicated that rBeta1 did not accelerate the gating current activation of hKv1.3, but shifted the Q-V in the depolarizing direction and slowed their kinetics. Because there are several differences in the amino acid sequences of mKv1.3 and hKv1.3, the differences between the previous report and our results may give clues on the molecular mechanisms of the functional interaction between Kv1.3 and rBeta1. Support: 13POST14800031 (AHA), U54GM087519 and R01GM030376

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The Effects of Auxiliary Subunits on Kv2.1 Pharmacology

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Voltage-gated potassium channels (Kv) are transmembrane proteins that respond to changes in membrane potential and regulate the flux of potassium ions across the cell membrane. It has been long known that auxiliary proteins that associate with voltage-gated ion channels can modify the channel in a number of ways, including cell-surface expression, voltage-dependent activation and inactivation, and pharmacology. Recent studies identified AMIGO-1 (amphoterin-induced gene and open reading frame) as an auxiliary subunit for Kv2.1 that increases the surface expression of the channel. Moreover, the presence of AMIGO alters the voltage dependency of activation of the channel (Peltola et al, 2011), suggesting a possible interaction site with Kv2.1 voltage sensors. These structural motifs are a well-known target of gating-modifier toxins isolated from venomous animals. Here, we investigate the role of AMIGO on the pharmacology of Kv2.1 expressed in Xenopus laevis oocytes. We hypothesize that AMIGO forms interactions with the voltage-sensing domains of Kv2.1, and thereby alters the binding affinity of the toxins. We find that the presence of AMIGO causes the channel to open at more hyperpolarized voltages, and influences the ability of gating modifier toxins to bind and inhibit the channel.

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Characterization of BK Channels Cloned from Mouse Sinoatrial Node Cells

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BK currents are well-established modulators of neural and smooth muscle excitability and recently we reported that BK currents alter the excitability of mouse sinoatrial node (SAN) cells, the predominant cardiac pacemaking cells